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JUL 2 8 1997

CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento CA 95814

Research Proposal Entitled
"A Program to Monitor Indicators of Stress in Fish for Adaptive Management of Aquatic Habitat"
RFP: 1997 Category III Ecosystem Restoration Projects and Programs
Principal Investigator - J.J. Cech

Dear Colleague:

It is our pleasure to present for your consideration the referenced proposal in response to **the CALFED Bay-Delta Program RFP.**

Please call on the principal investigator for scientific information. Administrative questions may be directed to me or my assistant, René Domino, at the above address and phone number. We request that correspondence pertaining to this proposal and a subsequent award be sent to the Office of Research and to the principal investigator.

Sincerely,

Sandra M. Dowdy

Sandra M. Dowdy
Contracts and Grants Analyst

Enclosure

cc: J.J. Cech

Item 8

ONDISCRIMINATION COMPLIANCE STATEMENT

PANY NAME	THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, disability (including HIV and AIDS), medical condition (cancer), age, marital status, denial of family and medical care leave and denial of pregnancy disability leave.

CERTIFICATION

I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.

L'S NAME	Sandra M. Dowdy Contracts and Grants Analyst		
ECUTED	JUL 28 1997	EXECUTED IN THE COUNTY OF	YOLO
TIVE CONTRACTOR'S SIGNATURE	Sandra M. Dowdy		
TIVE CONTRACTOR'S TITLE			
TIVE CONTRACTOR'S LEGAL BUSINESS NAME	THE REGENTS OF THE UNIVERSITY OF CALIFORNIA		

F1-037

A PROGRAM TO MONITOR INDICATORS OF STRESS IN FISH
FOR ADAPTIVE MANAGEMENT OF AQUATIC HABITAT

DR. JOSEPH J. CECH, JR.
UNIVERSITY OF CALIFORNIA, DAVIS

A PROPOSAL TO THE CALFED BAY-DELTA PROGRAM

JULY 1997

I. EXECUTIVE SUMMARY

a. Project title and applicant name: **A Program to Monitor Indicators of Stress in Fish for Adaptive Management of Aquatic Habitat; Dr. J. J. Cech, Jr., Professor, UC Davis.**

b. Project Description and Objectives: This multi-year project will combine field, laboratory and modeling work to develop improved monitoring methods for adaptive management of the Bay/Delta and associated riverine ecosystems, and will provide an evaluation of river conditions for juvenile chinook salmon during the study period. The program will complement more traditional monitoring of population levels with measures of condition and performance: long-term growth rates (from otoliths); short-term growth rates (from RNA/DNA ratios); energy stores (from lipids), readiness for seawater (Gill $NA^{+}K^{+}$ATPase activity); exposure to temperature stress or toxins (hsp 70); gross organ condition (field autopsy); and stomach contents. Field samples of juvenile fall-run chinook salmon will be collected with attention to spatial and temporal distributions. Laboratory studies of juvenile chinook and steelhead will (1) provide data on the measures of condition and their variation in fish raised in known conditions, and (2) develop cause-and-effect relations between the measures of condition and stressors such as water temperature and limited food supply. Individual-based models will be used to explore the importance of variation in the measured parameters for survival and reproductive capacity (starting in the second year). The utility of simpler v. more complex and expensive measures of condition will be compared, to allow design of an optimal long-term monitoring program. The initial emphasis of field studies will be on fall-run chinook salmon, to minimize concerns with sampling mortality during program development, but the method can be applied directly to other runs, and the basic approach can be extended to other fishes or invertebrates as well.

c. Approach/Tasks/Schedule: Field samples will be obtained in 1998, 1999, and 2000 from late January to late June in the Sacramento, American, Stanislaus and lower San Joaquin rivers and from the Delta, from existing sampling programs; additional samples will be collected in restoration sites or other habitats of particular interest. Environmental data will be obtained mainly from existing monitoring programs. The samples will be assayed for the measures condition and performance listed above. Laboratory studies in 1998 will be an expansion of a separately funded growth-temperature-ration study of juvenile chinook salmon and steelhead, and a simulation of the temperature stress experienced by chinook salmon smolts in the lower Sacramento River. The same measures of condition and performance will be measured for the experimental and control fish, to obtain data from fish held in known conditions. Laboratory studies in subsequent years will be designed based on results from the first year and from the field. Modeling studies (beginning in the second year) will explore the significance of variation in individual-based measures of condition and performance for the survival and reproductive capacity of individuals, and accordingly their significance for populations.

Local biologists and managers will be consulted individually in the first year, and a two-day workshop with local and invited biologists will be organized in the second year, to obtain peer review and ensure that the program addresses questions of concern to managers. Annual reports will be submitted to CALFED and if possible presented at the annual IEP meeting at Asilomar, in addition to quarterly progress reports.

Measures of condition and performance will be related to environmental conditions at capture sites, and to experimental conditons. Analyses of the data will consider statistical properties of the data (e.g., effect size and natural variation), and will also consider the information gained from more complex and expensive assays, compared to that obtained from simpler and less expensive measures. For example, the information gained from analysis of lipids into classes will be compared with the information gained from simple ether extraction of non-polar lipids. The final products in the year 2000 will be: (1) a report making recommendations for a long-term monitoring program; and (2) a report presenting, synthesizing and evaluating the results obtained from the field, modeling, and laboratory studies.

d. Justification: CALFED recognizes that adaptive management requires effective monitoring and has encouraged proposals for monitoring. Unfavorable environments or contaminants impose chronic stresses on organisims that manifest themselves in the performance and condition of individuals before they effect populations (Adams, 1990). Measures of the performance and condition of individuals are more effective than population-based or physical parameters because they have better statistical properties, and because they provide better evidence of the mechanisms that control populations (Osenberg et al. 1994). For such reasons, Castleberry et al. (1996) urged that monitoring for effective adaptive management of instream habitats include individual-based measures of condition and performance, along with measures of populations.

e. Budget Costs and Third Party Impacts: First year costs are $242,900, mainly for personnel. Costs of collecting and processing field samples for years two and three are $248,000 and $260,500 (assumes 5% inflation), for a total of $775,788 over three years. Experimental costs in the first year are mainly covered by a separate grant from the U.C. Water Resouces Center. Costs of experimental and modeling work in years two and three are estimated at $60,000 per year, but will be the subject of subsequent proposals. Hence, the estimated total cost is $907,749 over three years, and the **cost for this proposal is $775,788 over three years.** Salaries of the Principal Investigator and cooperating senior scientists will be paid by their institutions. No third party impacts are anticipated.

f. Applicant Qualifications: Dr. Joseph J. Cech is a professor at the University of California at Davis and a well recognized authority on the physiology and ecological physiology of fishes who has published over 80 peer-reviewed articles.

g. Monitoring Data and Data Evaluation: Monitoring is a primary objective of this proposal. Data analysis will relate measures of performance and conditon to environmental variables, and will use standard statistical methods appropriate for publications in professional journals. The biological significance of observed effects will be evaluated with bioenergetic and individual-based models.

h. Local Support/Coordination With Other Programs/ Compatibility With CALFED Objectives: The project will be coordinated with a similar National Marine Fisheries Service monitoring program covering the area from Chipps Island to the Gulf of the Farallones, with ongoing evaluations of the effectiveness of past restoration projects, and with regular agency sampling of juvenile salmon. This proposal will further CALFED objectives by developing and testing improved monitoring methods that will facilitate adaptive management.

II. TITLE PAGE

a. Title of Project: **A Program to Monitor Indicators of Stress in Fish for Adaptive Management of Aquatic Habitat.**

b. Names of Principal Investigator: Dr. Joseph J. Cech, Dept. of Wildlife, Fisheries and Conservation Biology, University of California, Davis, CA 95616, 916-752-3103, (fax) 916-752-4154, jjcech&ucdavis.edu; Professor, UC Davis.

c. Type of Organization and Tax Status: State Agency (University of California)

d. Tax Identification Number: 94-603-6494

e. Technical and Financial Contact Persons:
Technical: Dr. J. J. Cech, Jr., at the address above.
Financial: Ms. Marjorie Kirkman, Dept. of Wildlife, Fisheries and Conservation Biology, University of California, Davis, CA 95616, 916-752-6584, (fax) 916-752-4154, makirkman@ucdavis.edu.

f. Collaborators in Implementation:
Dr. R. Bruce MacFarlane, National Marine Fisheries Service, Tiburon, CA;
Dr. Michael K. Saiki, Biological Resources Division, USGS, Dixon, CA;
Dr. Jerry L. Hedrick, Molecular and Cellular Biology, UC Davis;
Dr. James S. Clegg, Bodega Marine Laboratory and UC Davis;
Mr. Daniel T. Castleberry, US Fish and Wildlife Service, Stockton, CA.
Dr. James Petersen, Biological Resources Division, USGS, Cook, Washington.

g. RFP Project Group Type: Other Services

III. PROJECT DESCRIPTION

a. Project Description and Approach: This three year project will combine field, laboratory and modeling work to develop improved monitoring methods for adaptive management of the Delta and associated riverine ecosystems. Field samples of juvenile fall-run chinook salmon will be assayed for the following measures of condition and performance: long-term growth rates (from otoliths); short-term growth rates (from RNA/DNA ratios); energy stores (from lipids); readiness for seawater (Gill NA^+K^+ ATPase activity); exposure to temperature stress or toxins (hsp 70); and gross organ condition (field autopsy). Stomach contents will also be analyzed. Laboratory studies will develop cause-and-effect relations between the measures of condition and stressors such as water temperature, which will allow more robust interpretation of the field data. Individual-based models will explore the importance of variation in the measured parameters for survival and reproductive capacity, linking the field data to population consequences.

To allow design of an optimal long-term monitoring program, the utility of simpler measures of condition will be compared to the utility of more complicated and expensive measures. Managers and agency biologists will be consulted to identify specific questions of management concern, and other research scientists will be consulted to identify other potential measures of condition and performance, as well as any technical difficulties with the proposed assays and interpretation of results. The initial emphasis will be on fall-run chinook salmon, to minimize concerns with sampling mortality during the developmental stage of the program, but the approach can be applied directly to other runs, and the basic approach can be extended to other fishes as well. For example, RNA/DNA ratios can be obtained from larval striped bass (Heath et al. 1993), and could be used to investigate the influence of flow on their growth.

b. Geographic boundaries of the project: Field samples will be collected in the Sacramento River from Balls Ferry downstream, the American River, the Stanislaus River, the lower San Joaquin River, and at several sites in the Delta including Chipps Island. Laboratory studies will be conducted at UC Davis.

c. Expected benefits:
The primary benefits from this project will be (1) a protocol for monitoring the condition and performance of juvenile salmonids in the Delta/rivers system, (2) an analysis of three years of monitoring data, supported by laboratory experiments and modeling, documenting the condition and performance of juvenile fall-run chinook salmon in the study area, and (3) improved understanding of the temperature tolerance of juvenile chinook salmon and steelhead.

The monitoring is intended to address specific questions of management interest such as:

What is the relation between water temperature and the growth and condition of juveniles rearing in the Sacramento-San Joaquin river system?
What is the relation between water temperature and the condition of smolts captured in the Delta?
How does the condition of fry or smolts change as they emigrate through the system?
Is there evidence of food shortages for juveniles in the lower Sacramento or San Joaquin rivers?

- Is there evidence of stress induced by contaminants?
- Do short term growth rates of juveniles differ in different habitats in the Delta, with particular attention to restoration projects?
- Does the growth and condition of juvenile fall-run chinook salmon within given rivers or habitats vary with short-term or seasonal differences in discharge?
- Does the condition of emigrating chinook fry differ from that of fry that rear to smolt size before emigrating?

The stressors targeted by the proposal are water temperature, water quality (contaminants), and hydrograph alterations. The targeted species are fall-run chinook salmon (including San Joaquin River fall-run) and steelhead, but once the protocol is developed it could be extended to winter-run chinook salmon and spring-run chinook salmon if the anticipated benefits would outweigh the associated sampling mortality. The basic approach can be adapted to other fishes or invertebrates. The monitoring approach is intended especially for adaptive management of instream aquatic habitat, but can also be applied to tidal perennial aquatic habitat, saline emergent wetlands, and shaded riverine aquatic habitat.

d. Background and Biological/Technical Justification:
In a study monitoring the environmental effects of off-shore oil production in the Santa Barbara Channel, Osenberg et al. (1994) found that individual-based parameters were more effective than population-based or physical parameters because they had better statistical properties, and because they provide better evidence of the mechanisms that control populations. Castleberry et al. (1996) urged that individual-based measures of condition and performance be monitored, in addition to population measures, for effective adaptive management of instream habitats. The measures proposed for monitoring are described below.

- Otoliths: Because salmonids generally form one otolith growth increment each day (Bradford and Geen 1987; Campana 1983; Neilsen et al. 1985; Castleberry 1994), the number of otolith increments divided by length provides an index of long-term growth rate, and if a length is assumed at formation of the first increment or some other identifiable increment, the subsequent absolute growth rate can be estimated. Increment widths are also closely related to growth rate over extended periods (e.g. 50 days), although not for shorter periods (Bradford and Geen 1987).

- RNA/DNA ratios: RNA/DNA ratios provide a measure of short-term growth rates, because the amount of RNA in cells increases during active growth but the amount of DNA is relatively constant. However, the relation between the ratio and growth rates depends upon temperature (Buckley 1982, Ferguson and Danzmann 1990). We will follow the example of Buckley (1984), who used data from larval fish reared at different temperatures in the laboratory to estimate growth rates of larval fish at sea based on water temperature and RNA/DNA ratios. A measure of short-term growth rate will be useful for evaluating the utility of specific habitats for juvenile salmon; rapid growth rates would be more powerful evidence of benefit to juvenile salman than their mere presence.

Lipids: Lipids are an important source of potential energy that reflects the physiological capacity of fish for growth or activity (Busacker et al. 1990), and lipid levels of juvenile salmonids from hatcheries affect their survival (Peterson 1973, Rondorf et al. 1985, Wagner 1974). Castleberry et al. (1991; 1993) analyzed lipid levels for juvenile chinook salmon and steelhead from the American River for two similar years (1991 and 1992), and found the levels to correspond with the lower range of levels for fed fish in hatcheries or laboratory experiments. However, few other data are available on lipid levels in naturally produced juvenile salmonids in rivers. Lipids occur in various forms. Castleberry et al. reported data for "non-polar" lipids, which can be determined by a simple ether extraction technique. Chromotographic analysis allows a more detailed and potentially more informative breakdown of lipids into sterol/wax esters, triacylglycerol, nonesterified fatty acids, cholesterol, and polar lipids (MacFarlane and Norton 1996), and is being used in an ongoing study of salmon smolts from Chipps Island to the Gulf of the Farallones by Dr. Bruce MacFarlane of the Tiburon Laboratory, National Marine Fisheries Service. We will use both methods concurrently, in order to assess whether the extra information provided by the chromotographic assay justifies the extra cost.

Gill Na^+K^+ ATPase activity: Gill sodium, potassium-activated adenosine triphosphate levels have been used as a quantitative measurement of the progress of smoltification of migrating salmon in routine monitoring on the Columbia River (Beeman et al. 1991). The enzyme is used in the transport of NaCl across the gill epithelium, which is necessary for the survival of salmon smolts in salt water. There is concern that temperature stress may lead to reduced ATPase activity (Zaugg 1981), which could further compromise salmon smolts migrating through the Delta. ATPase assays will be concentrated on fish sampled from the Delta and the lower reaches of the Sacramento and San Joaquin rivers, and from the American River. Assays of the ATPase activity of fish exposed in the laboratory to thermal stress simulating migration through the lower Sacramento River in unfavorable conditions will provide a reference from known conditions.

Stress proteins (hsp 70): Exposure to thermal or other stresses induces synthesis of small "heat shock " proteins, particularly members of the hsp 70 family (Morimoto et al. 1990 and 1994; Feige et al. 1996). Although the functions of heat shock proteins continues to be elucidated it is known that some, including the hsp-70 family, function as molecular chaperones that that reduce damage to other proteins, and account for acclimation to warmer water (Mosser and Bols 1988). Smolting fall-run chinook salmon are frequently subjected to high water temperatures in the lower Sacramento and San Joaquin rivers. Previous attempts to relate smolt survival to water temperature have used paired releases of coded-wire tagged fish. Although the data have been given competent statistical analysis (Baker et al. 1995) considerable uncertainty remains. Assays of one of these "heat shock" proteins, hsp 70, would provide an independent measure of the temperature stress that fish experience. Hsp 70 synthesis is also induced by other stressors such as various toxins that, like high temperatures, act by altering the biologically effective three-dimensional geometry of proteins. Accordingly, the presence of hsp 70 that cannot be accounted for by temperature stress indicates exposure to such stressors.

Field dissection: Sublethal stresses such as low levels of contaminants commonly induce changes in the color or gross appearance of organs and tissues that can be detected by field necropsy, and protocols have been developed for recording these systematically to obtain a profile of the health of a fish population based on percentages of observed anomalies (Goede and Barton 1990; Foott 1990, Adams et al. 1993).

Stomach Contents: Recently ingested prey provide a measure either of habitat quality, or of the ability of the fish to feed successfully. For example, terrestrial insects in stomachs

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provide evidence of the importance of riparian vegetation as a source of food. The amount of food in stomachs of recently emerged fry captured in screw (outmigrant) traps provides evidence about the viability of early-emigrating fry.

e. Proposed Scope of Work:
Field Studies: Samples will be obtained from existing monitoring programs in the Sacramento-San Joaquin river systems. Tentatively, we plan to obtain samples of juvenile salmon from trawl catches at Chipps Island, Mossberg and Sacramento, from floating rotary traps on the Stanislaus, American and Sacramento rivers (Knights Landing, Red Bluff, and Balls Ferry), and from USFWS seining in the lower Sacramento River and Delta (Table 1). Samples will be obtained every two weeks from late January to late June. Additional seine samples of presumably non-emigrating juveniles will obtained by seining along the edge of the lower American River near the upstream limit of salmon spawning. If possible, samples will also be obtained from ongoing evaluations of habitat restoration sites in the Sacramento-San Joaquin river Delta. Environmental data will be obtained mainly from existing monitoring programs, but spot measurements will be taken at field sampling sites for backup and quality assurance.

Field and analytical procedures will be generally similar to those of Castleberry et al. (1991; 1993), with minor changes based on subsequent reports in the literature (e.g. McGurk and Kusser (1992) regarding RNA/DNA ratio determination). After capture, samples of up to 40 fish will be over-anesthetized (MS-222, 250 mg/l), measured to the nearest millimeter for standard and fork lengths, and weighed to the nearest 0.1 gram. Up to 15 fish will be selected for determination of lipid levels, placed in individually numbered sample bags and frozen on dry ice. Selected samples (approximately 1/4th) will be analyzed for total lipids and lipid classes (triacylglycerols, sterol/wax esters, nonesterified fatty acids, cholesterol, and polar lipids) using thin layer chromatography with flame ionization detection at the National Marine Fisheries Service Laboratory in Tiburon, with the assistance of Dr. Bruce MacFarlane. The remaining samples will be analyzed for non-polar lipids by ether extractions of lipids and consequent changes in weight (dry weight - lipid-free dry weight). Up to 15 other fish will be placed on ice and dissected in the field. After the condition of organs is noted, tissues will be removed for other assays and placed in numbered containers (one number per fish), and then frozen on dry ice. The subsamples of 15 will be selected to have approximately equal size distributions. Up to 10 additional fish will be frozen on dry ice and archived for future analysis. Samples will be taken promptly to the laboratory and stored at -80°C until they are processed. Non-polar lipid extractions, stomach content analyses, and determination of RNA/DNA ratios will be done at UC Davis. Otoliths will be processed at UC Davis and by UC Davis personnel at the USGS Dixon lab, with the assistance of Dr. Mike Saiki. Hsp 70 assays will be conducted at the Bodega Marine Laboratory under the direction of Dr. James Clegg. Gill $Na^{+}K^{+}$ATPase activities will be determined by Biotech Research and Consulting, Inc., in Corvalis, Oregon.

Laboratory Studies: laboratory studies in the first year (1998) will take advantage of an ongoing study of the relations among growth, temperature and ration level for juvenile chinook salmon and steelhead, which has separate funding. Funding under this proposal will allow assays of lipid classes, gill ATPase activity, RNA/DNA ratios, and stress proteins (hsp 70), to provide data on these measures of condition and performance for fish grown in known conditions. In addition, separate experiments will be conducted on chinook smolts from Nimbus Hatchery, to simulate thermal conditions experienced by smolts emigrating through the lower Sacramento River.

Experiment 1: Fish will be held at the UC Davis Institute of Ecology, and will be randomly assigned to one of the 6 temperature (11, 15 and 19°C) x ration size (100% ad libitum and 30% of ad libitum) combination treatments, with 4 replicate tanks per treatment. This represents a total of 48 tanks, the maximum number available in the facility. Response variables in Experiment 1 will be: (1) food consumption, growth and mortality rates; (2) respiratory metabolic rates; (3) swimming endurance determined by critical swimming velocity; (4) critical thermal maxima; (5) thermal preference; and (6, this proposal) lipid levels and composition, RNA/DNA ratios, ATPase activities, and stress protein (hsp 70).

Juvenile steelhead and chinook salmon will be maintained in replicate round, 75-l, fiberglass experimental tanks. A flow-through, temperature-controlled unchlorinated well-water supply with continuous aeration will assure high dissolved oxygen and low dissolved ammonia concentrations, both of which will be regularly monitored. Spray bars carrying inflowing water will be angled to produce a slow current of approximately 1 body length per second. Tanks will be situated in a laboratory equipped with translucent roof panels to maintain natural photoperiods throughout the experiment. Baseline data will be collected from a sample of 30 fish selected randomly at the beginning of the experiment and over-anesthetized for determination of lipid levels and composition, ATPase activity, and levels of stress protein (hsp 70). A subsample of 15 will be selected for determination of lipid classes. The second subsample of 15 will used for determination of RNA/DNA ratios ATPase activity, and hsp 70 levels. Procedures and methods will be as described above for field samples. At the conclusion of Experiment 1 as second sample of up to 30 fish, possibly including fish used in the thermal preference tests, will be selected from each treatment group for the same set of assays.

Experiment 2: After the conclusion of Experiment 1, juvenile chinook salmon will be obtained from Nimbus Hatchery and held at 17°C until at least half display color changes (silvering) associated with smolting. Fish will then be starved for 24-48 hours to empty the gastrointestinal tracks, and a sample of 30 will be selected for the same baseline assays as described above for Experiment 1. After the fish are allowed to recover for two days, they will be divided randomly into six groups of 36, and each group will be distributed among four of the tanks used in Experiment 1, giving nine fish per tank. Water temperature in 1/3rd of the tanks will be increased to 20°C, to 22°C in another third, with the final third of the tanks remaining at 17°C. Within each temperature treatment, one group will be feed ad libitum, and the other group will be fed 30% of the amount consumed by the ad libitum group, for six days. Dead fish will be promptly removed from the tanks, counted, weighed, and measured.

After seven days (the approximate travel time from Sacramento to Chipps Island; Pat Brandes, pers. comm.) fish will be over-anesthetized, weighed and measured as in Experiment 1, and fish from each experimental group will be divided into two subsamples with approximately equal size distributions. Lipid classes will be determined for one subsample, and the same methods as in Experiment 1. Tissues will be taken from the other subsample for assays of ATPase activities, RNA/DNA ratios, and levels of stress protein (hsp 70). Methods and procedures will be as described above. Mortality will be directly measured as incidental deaths.

Modeling Studies:
Modeling studies will begin in the second year. The models used should be matched to the data, so data from the first year will be used to guide selection or development of the models. During the first year existing models will be collected and evaluated. We anticipate using models that will explore the population consequences of differences in performance and condition in plausible and relevant but gerneralized environmental conditions; that is, the focus will be on modeling the responses of fish to environmental conditions, rather than on modeling the environmental conditons.

Workshop: We will organize a workshop in 1999 to get mid-course peer review from invited scientists working on related issues. Tentatively, we plan that the first day of the workshop would include managers and biologists from the Central Valley for presentations of our first year results and discussion of the utility of the work presented for addressing practical management problems. The second day of the workshop would be a more technical review of the results from the first year, and also deal with such matters as alternative measures of condition and performance, analytical techniques for assays, and discussion of future lines of research.

Data analysis and synthesis:
Analyses of laboratory date will use standard statistical methods such as ANOVA. Analyses of field data will also use standard statistical methods, but as recommended by Stewart-Oaten (1996) will emphasize confidence intervals rather than significance tests. Archived fish will be processed as needed to provide additional statistical power. Methods such as cannonical ordination (Jongman et al. 1987) will also be explored. Data from the National Marine Fisheries Service monitoring program will be included in analyses to the extent feasible and appropriate. We will also merge and analyze the 1991 and 1992 American River data on chinook salmon and steelhead that were reported separately in Castleberry et al. (1991, 1993).

Products: The products of this project will be (1) a report making specific recommendations for a long-term monitoring program; (2) a substantial set of data on the condition of juvenile fall-run chinook salmon; (3) a report presenting, synthesizing and evaluating the results obtained from the field, modeling, and laboratory studies; (4) improved understanding of the temperature tolerance of juvenile chinook salmon and steelhead. The work will also be reported in the professional literature.

f. Monitoring and Data Evaluation:
The monitoring program is specifically designed to be coordinated with existing programs for monitoring juvenile salmon, including the NMFS program. The workshop in proposed for 1999 will provide peer review from both scientific and management perspectives; in addition, manuscripts describing results of the program will be submitted for publication in peer-reviewed journals. Data will be evaluated both by statistical analyses and by modeling, as described above.

g. Implementability:
The proposed program will be highly implementable regarding fall-run chinook salmon, although there may be constraints on field sampling of juvenile steelhead or juvenile chinook from other runs. All necessary permits will be obtained. The cooperation of agency personnel conducting the existing sampling programs will be required.

IV. COSTS AND SCHEDULE

a. Budget Costs:
This proposal is for $779,500 over three years, with $247,300 in the firt year. Costs are mainly for salaries of post-graduate researchers ($497,870), with adjustments for inflation of 5% per year included. Students will be employed on a hourly basis to help with specific tasks, but the project depends primarily on salaried personnel. The estimated total cost of the program is $879,500 over three years, of which an estimated $120,000 for modeling and experimental work will be the subject of future proposals. As the routine for processing samples becomes well developed, it may be possible to reduce costs; such adjustments in the cost of processing samples will be included in the future proposals.

Costs for are broken down into the requested categories, by year, in Table 1. Costs in 1998 for processing samples, excluding salaries of post-graduate researchers, are broken down by assay in Table 2. 5% increases are assumed for 1999 and 2000.

Potential for incremental funding: Because the design of experiments and modeling in the second and third years should depend upon results in prior years, we will submit separate proposals for that work. It would be possible to fund the project one year at a time, with a first year budget of $247,300; however, interpretation of the field results will depend strongly on year-to-year variation, so a three year program seems minimal. Moreover, a multi-year commitment will facilitate attracting top-quality post-graduate researchers and maintaining a smoothly running program.

b. Schedule Milestones:
Milestones:

December 1997:	Funding begins; Organize for field sampling, train post-graduate researchers in analytical techniques as needed;
January 1998:	Begin field collects;
April 1998:	Begin laboratory experiments;
June 1998:	Conclude laboratory experiments and first year field collections.
mid-1998:	Submit proposal for 1999 laboratory experiments and laboratory work.
November 1998	Submit annual report for 1998, begin modeling studies;
January 1999:	Begin second-year field collections and experiments; Hold workshop;
June 1999:	Conclude second-year field collections and experiments;
November 1999:	Submit annual report for 1999;

January 2000:	Begin third-year field collections and experiments;
June 2000:	Conclude third-year field collections and experiments;
November 2000:	Final report completed, funding ends.

c. Third Party Impacts:
No third party impacts are anticipated.

V. APPLICANT QUALIFICATIONS:

a. Organization of staff:
The project will be under the direction and supervision of Dr. J. J. Cech, Jr., Professor at the Department of Wildlife, Fish, and Conservation Biology, University of California, Davis. Dr. J. G. Williams (Post Graduate Researcher X) will provide day to day project management, be primarily responsible for data analysis and report writing, and participate in the modeling work. Ms. Barbara Martin (PGR III) will be primarily responsible for the preparation and analysis of otoliths, for conducting the lipid class analyses, and for supervising the RNA/DNA analyses. One PGR II will be responsible mainly for conducting the hsp 70 analyses. Another PGR II will be responsible for collecting field samples and performing the field autopsies, during the field season, and will assist with the otolith and RNA/DNA analyses during the balance of the year. We anticipate that a PGR V (Ph.D. level) will be included in the year 2 and 3 proposals to conduct laboratory experiments and participate in the modeling work.

b. Collaborating Scientists:
Dr. Bruce MacFarlane of the National Marine Fisheries Service, Tiburon, California, will provide guidance for the lipid class analyses performed in his laboratory by Ms. Barbara Martin, and will collaborate in the interpretation of data, particularly with the integration of results from this proposal with the results of his similar investigations in San Francisco Bay and the Gulf of the Farallones.

Dr. Michael K. Saiki of the Biological Resources Division of the U.S. Geological Survey in Dixon will make facilities and equipment available for otolith analyses, and will collaborate particularly on indications of effects of contaminants, which is his area of specialization.

Dr. James S. Clegg will provide guidance for the hsp 70 assays, which will be conducted in his laboratory at the University of California Bodega Marine Laboratory.

Dr. James Peterson of the Biological Resources Division of the U.S. Geological Survey at Cook, Washington, will collaborate on the modeling studies.

Dr. Jerry Hedrick of the University of California, Davis, will make facilities and equipment available for the RNA/DNA analyses.

Mr. Daniel T. Castleberry of the U.S. Fish and Wildlife Service in Stockton will collaborate with the interpretation of the study results, and with the synthesis of the results of this project with those from his work on the American River with Dr. Cech, Dr. Saike, and Ms. Martin.

c. Biosketches:
Dr. J.J. Cech Jr. has been a professor at the University of California, Davis, since 1975, and was Chair of the Department of Wildlife, Fisheries, and Conservation Biology from 1992-1997. He has published over 80 peer-reviwed articles and books in the fields of physiology and physiological ecology of fishes, and has won numerous award, honors, and grants. He has successfully completed seven contracts with state agencies for studies of the physiological ecology of fish in the Sacramento/San Joaquin/Delta system, and participated

in studies of the condition and performance of juvenile chinook salmon and steelhead in the American River. Relevant publications include:

Cech, J.J., Jr., Mitchell, S.J., Castleberry, D.T., and McEnroe, M. 1990. Distribution of California stream fishes: influence of environmental temperature and hypoxia. Env. Biol. Fish. 29:95-105.

Castleberry, D.T. and J.J. Cech, Jr. 1993. Critical thermal maxima and oxygen minima of five fishes from the Upper Klamath Basin. Calif. Fish and Game 78:145-152.

Young, P.S. and J.J. Cech, Jr. 1993. Improved growth, swimming performance, and muscular development in exercise-conditioned young-of-the-year striped bass (*Morone saxatilis*) Can. J. Fish. Aquat. Sci. 50:703-707.

Young, P.S. and J.J. Cech, Jr. 1993. Effects of exercise conditioning on stress responses and recovery in cultured and wild young-of-the-year striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 50:2094-2099.

Cech, J.J. Jr., D.T. Castleberry, T.E. Hopkins, and J.H. Petersen. 1994. Northern squawfish, *Ptychocheilus oregonensis*, O_2 consumption and respiration model: effects of temperature and body size. Can. J. Fish. Aquat. Sci. 51:8-12.

Cech, J.J., Jr., D.T. Castleberry, and T.E. Hopkins. 1994. Temperature and CO_2 effects on blood O_2 equilibria in squawfish, *Ptychocheilus oregonensis*. Can. J. Fish. Aquat. Sci. 51:13-19.

Moyle, P.B. and J.J. Cech, Jr. 1996. Fishes: an introduction to ichthyology. 3rd ed., Prentice Hall.

Cech, J.J., Jr., S.D. Bartholow, P.S. Young, and T.E. Hopkins. 1996. Striped bass exercise and handling stress in freshwater: physiological responses to recovery environment. Trans. Am. Fish. Soc. 125:308-320.

Young, P.S. and J.J. Cech, Jr. 1996. Environmental tolerances and requirements of splittail. Trans. Am. Fish. Soc. 125:664-678.

Dr. J.G. Williams is a hydrologist who became familiar with fisheries issues during his tenure as Special Master for the continuing jurisdiction in *Environmental Defense Fund et al. v. East Bay Municipal Utilities District*, in which capacity he supervised a major monitoring program on the American River. He also had experience with project management while working with the firm of Philip Williams & Associates prior to his American River work. He recently became Executive Director (part-time) of the Bay Delta Modeling Forum.

Ms. Barbara Martin is now completing a Master's Degree at Humboldt State University. From 1991 to 1993 she participated in studies of the condition and performance of juvenile salmon and steelhead in the American River (Castleberry et al. 1991, 1993, 1994), where she had extensive experience in preparing and reading otoliths, and conducting RNA/DNA analyses, as well as performing other assays.

VI. COMPLIANCE WITH STANDARD TERMS AND CONDITIONS

All terms and conditions are agreeable and we will be able to comply with them. An interagency agreement will be executed along with the final contract.

COST BREAKDOWN BY CATEGORY

Task:	Salary and Benefits	Hourly Labor	Service Contracts	Miscellaneous and other direct costs	Overhead	
YEAR ONE						
Collect Field Data	20,969[1]	1,000[2]	--	4,000[3]	2,597	
Conduct Lab Experiments				2,000[4]	200	
Process Samples	108,831[5]	10,085[6]	13,875[7]	33,985[8]	16,678	
Data Analysis, Project Management	30,137[9]	--	--	--	3,014	
YEAR TWO						
Workshop	--	--	--	4,000[11]	400	
Collect Field Data	22,017[1]	1,050[2]	--	4,200[3]	2,727	
Conduct Lab Experiments[10] and modeling	*50,227*	--	--	*8,300*	*5,853*	
Process Samples	114,273[5]	10,589[6]	14,569[7]	35,684[8]	17,511	
Data Analysis, Project Management	31,643[9]	--	--	--	3,164	
YEAR THREE						
Collect Field Data	23,199[1]	1,103[2]	--	4,441[3]	2,874	
Conduct Lab Experiments[10] and modeling	*52,738*	--	--	*8,700[4]*	*6,144*	
Process Samples	119,987[5]	11,118[6]	15,297[7]	33,985[8]	18,039	
Data Analysis, Project Management	33,225[9]	--	--	--	3,323	
Totals	607,246	34,945	43,741	139,295	82,523	907,749
THIS PROPOSAL: *(less estimated year 2 and 3 costs)*	504,281	34,945	43,741	122,295	70,526	775,788

Notes on following page.

TABLE 1

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Notes for Table 1:

(costs in notes are for Year One, add 5% for subsequent years)

1. One Post Graduate Researcher II (PGR II) for 6 months
2. Student labor for seine sampling in the American River and the Delta.
3. Materials, supplies, and travel
4. Modify water supply for temperature control for Experiment 2.
5. One PGR III for 12 months, one PGR II for 12 months, one PGR II for 6 months.
6. Student labor at $5.75 per hour for identifying stomach contents and preparing samples for lipid extractions.
7. Processing ATPase samples, 6,975 (Biotech Research and Consulting, Inc.); lipid extractions, 7,080 (Dept. of Animal Science).
8. Supplies for analyses: lipid class determinations, 15,375; hsp 70 assays, 4,050; otoliths, stomachs, and common to all, 3,085.
9. One PGR X for 12 months at 50% time.
10. *Estimates: will be the subject of future proposals.*
11. Travel costs for invited scientists, facilities.

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ANALYSIS COSTS
1998

Cost per sample	(includes labor but) not salaries	Sample Size	No. Samples	Cost
Lipid classes	25	15	42	15,750
non-polar lipids	5	15	115	8,625
hsp 70	5	15	54	4,050
ATPase	5	15	93	6,975
RNA/DNA	5	15	153	14,475
otoliths	1	15	139	4,170
stomachs	3	15	139	6,255
				$60,300

TABLE 2

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